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A novel *FN1* variant associated with familial hematuria: TBMN?

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ABSTRACT

Objective: Thin basement membrane nephropathy (TBMN), an autosomal dominant inherited condition in general, is characterized clinically by persistent hematuria and pathologically by thinning of glomerular basement membrane. TBMN is occasionally accompanied with proteinuria, hypertension and renal impairment in some cases. The aim of this study is to explore the genetic defect in a Chinese pedigree with familial hematuria.

Design and methods: A four-generation Chinese Han pedigree with familial hematuria was recruited. Exome sequencing was conducted in the proband diagnosed as TBMN, followed by verification in the proband and other family members with Sanger sequencing.

Results: A novel missense variant, c.4616C>G (p.S1539C), in the fibronectin 1 gene (*FN1*), was identified, and it co-segregated with the disease condition in the family. It was not observed in 100 normal controls.

Conclusions: A missense variant in the *FN1* gene is possibly responsible for familial hematuria or TBMN in this family, which may broaden the phenotype and mutation spectrums of the *FN1* gene. A male patient in this family progressed to end-stage renal disease requiring kidney transplantation, supporting that familial hematuria or TBMN may not always be as benign as generally thought. The findings may have new implications for clinical monitoring and genetic counseling of the family, and may also help understand the pathogenesis.

1. Introduction

Thin basement membrane nephropathy (TBMN, MIM 141200), previously known as benign familial hematuria (BFH), is responsible for glomerular hematuria in at least 1% of the worldwide population, and is the most common cause of microscopic hematuria [1–3]. However, the term, BFH, is turned out to be a misnomer since it may not be as benign as commonly thought [4,5]. TBMN is described clinically by persistent microscopic hematuria and pathologically by diffuse or segmental thinning of the glomerular basement membrane (GBM) without typical lamellation or thickening of the GBM observed in Alport syndrome (AS) on ultrastructural examination [6–8]. In contrast to AS, significant proteinuria, hypertension, renal impairment, or extrarenal symptoms are not commonly seen in TBMN patients [4,9]. TBMN is mainly an inherited disorder with an autosomal dominant transmission, but the penetrance of hematuria is only 70% [2,8,10]. Approximately 40–50% of families with TBMN are reported to be caused by heterozygous mutations in the collagen type IV alpha-3 or alpha-4 gene (*COL4A3*/*COL4A4*), generally representing the carrier status of autosomal recessive AS, or attributed to heterozygous/hemizygous mutations in the collagen type IV alpha-5 gene (*COL4A5*) commonly associated with X-linked AS [2,5,11]. TBMN may be also viewed as an extreme in the spectrum of AS sharing similar clinical features, possible progression, genetic defects, and pathological changes [2,4,6,12]. Though the prognosis of TBMN is relatively favorable, there is also a raised risk of developing proteinuria, hypertension, or renal impairment in some patients, which are all risk elements for progression to end-stage renal disease (ESRD) [2,3]. Diagnosis of possible/probable TBMN is based on the thinning of GBM that involves at least 50% of the GBM, without lamellation generally seen in AS, while the definite diagnosis can be difficult. In general, TBMN cannot be differentiated from the primary stage of AS [2,4,13].

Because of the clinical and genetic heterogeneity in familial hematuria [14], we performed exome sequencing in a proband from a four-generation Chinese Han pedigree with hematuria to identify the genetic

Abbreviations: TBMN, thin basement membrane nephropathy; BFH, benign familial hematuria; GBM, glomerular basement membrane; AS, Alport syndrome; *COL4A3*, the collagen type IV alpha-3 gene; *COL4A4*, the collagen type IV alpha-4 gene; *COL4A5*, the collagen type IV alpha-5 gene; ESRD, end-stage renal disease; *FN1*, the fibronectin 1 gene; gDNA, genomic DNA; SNPs, single nucleotide polymorphisms; indels, insertions–deletions; ANNOVAR, Annotate Variation; ESP, exome sequencing project; SIFT, Sorting Intolerant from Tolerant; PolyPhen-2, Polymorphism Phenotyping version 2; BLAST, Basic Local Alignment Search Tool; SNAP, Screening for Non-Acceptable Polymorphisms; GFND, glomerulopathy with fibronectin deposits; EDA, extra domains A; EDB, extra domains B; IIICS, type III connecting segment.

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defect. A novel heterozygous c.4616C>G transversion (p.S1539C) in the fibronectin 1 gene (*FN1*) was identified and the variant co-segregated with the disease condition in the family.

2. Materials and methods

2.1. Pedigree and participators

A four-generation Chinese Han pedigree consisting of 10 individuals was enrolled in the study from the Third Xiangya Hospital, Central South University, P.R. China (Fig. 1). Clinical data and specimens of peripheral blood were obtained from six members of the pedigree, including three affected individuals (II:2, III:1 and III:3) and three unaffected members (II:3, III:2 and IV:1). Blood samples were also taken from 100 unrelated ethnically-matched normal controls (male/female: 50/50, age 38.5 ± 5.6 years). Written informed consent was signed by the participating individuals or their parents, and this study had received approval from the Medical Ethics Committee of the Third Xiangya Hospital, Central South University, P.R. China.

2.2. Clinical data

Detailed physical examinations, laboratory analyses, and genetic evaluation were performed in the available family members. The definition of affected status was made by two independent nephrologists based on the family history, clinical features and renal biopsy of the family members (Table 1). No evidence of other extrarenal abnormalities including hearing loss or ocular anomalies, or systemic disorders was found in the affected subjects [4,9]. Renal biopsy was performed in the proband (III:3). Pathological diagnosis was made by Department of Pathology of Xiangya Hospital, Central South University, P.R. China. The light microscopy of glomerular histology was almost normal and electron microscopy showed diffuse thinning of GBM, typical pathological feature of TBMN, without glomerular electron-dense deposits, which supported the diagnosis of TBMN in the proband. Kidney transplantation was performed in his brother (III:1) at the age of 48 with ESRD.

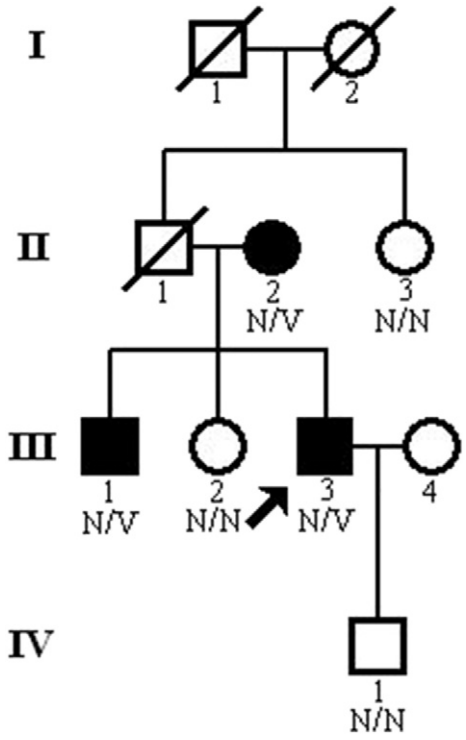

Fig. 1. Pedigree of the family with familial hematuria showing affected cases (fully shaded). N, normal; V, the *FN1* p.S1539C variant. The arrow indicates the proband with thin basement membrane nephropathy.

Table 1
Clinical data of three patients with heterozygous c.4616C>G (p.S1539C) variant in the *FN1* gene.

Subject	II:2	III:1	III:3
Gender	Female	Male	Male
Age (years)	74	52	45
Diagnosed age (years)	40	44	30
Microscopic hematuria	Yes	Yes	Yes
Proteinuria	Yes	Yes	No
Renal function	Normal	End-stage renal disease at 48 years old	Chronic kidney disease stage II
Hypertension	Yes	Yes	Yes

2.3. Exome capture

Peripheral blood genomic DNA (gDNA) was extracted from leukocytes using the phenol-chloroform extracting method [15]. Exome sequencing of gDNA from the proband (III:3) was performed by Novogene Bioinformatics Institute, Beijing, China. Paired-end DNA library was prepared following the manufacturer's directions (Agilent Technologies Inc., USA). 1.5 μg of gDNA was used to establish the exome library, and gDNA was randomly sheared into 180–280 bp fragments by a Covaris S220 sonicator. Exome capture was implemented using the Agilent's SureSelect Human All Exon V5 Kit. After quality assessment of DNA, pooled samples were prepared for sequencing. Sequencing of captured DNA library was performed on a HiSeq 2000 platform in accordance with the manufacturer's protocols (Illumina Inc., USA) [15,16].

2.4. Variant analysis

Quality control was applied to raw data (stored in FASTQ format) obtained from HiSeq 2000 to guarantee the meaningfulness of downstream analysis. High quality paired-end reads were aligned to the human reference genome sequence from UCSC database (build 37.1 version hg19, http://genome.ucsc.edu/) using Burrows-Wheeler Alignment tool [17]. To detect single nucleotide polymorphisms (SNPs) and insertions–deletions (indels), high quality alignment is required to guarantee variant calling accuracy (greater than 0). After duplicate removal, local alignment, and base quality recalibration by Picard (http://sourceforge.net/projects/picard/), Genome Analysis Toolkit and SAMtools, the analysis-ready BAM alignment results were obtained. The thresholds for calling SNPs included the alignment rate of sequencing reads ≥95% and the coverage of sequence depth ≥10× [18,19]. ANNOVAR (Annotate Variation) was used to annotate SNPs and indels, including variant position, variant type, and conservative prediction. Given that the variant causing Mendelian diseases is assumed to be rare in the public databases, variant filtrations were performed using data from the database of SNPs build 137 (dbSNP137, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), 1000 genomes project (2012 April release, http://www.1000genomes.org/), and NHLBI exome sequencing project (ESP) 6500, and a prioritization scheme similar to those in previous studies was utilized [15,16,20]. Variants retained after this step were considered to be “novel”. Only SNPs and indels occurring in exons or located in canonical splicing sites were further analyzed, and nonsynonymous SNPs were submitted to Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping version 2 (PolyPhen-2) for functional prediction [15,21].

Primers for locus-specific PCR amplification were designed based on the human genomic sequences (accessed from UCSC database), and primer specificity was measured by Primer-BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/tools/primer-blast/). To verify the potential disease-causing variant, PCR amplification from